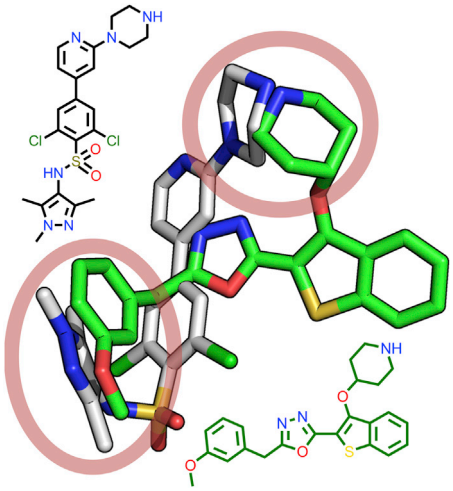
From Malaria to Worms

Each month, *Chemistry & Biology* Select highlights a selection of research reports recently published in other journals. These highlights are a snapshot of interesting research done across the field of chemical biology and this month's Select features a new study of *Plasmodium* N-myristoyltransferase (NMT), a discovery of an alternative cation conducting pathway in a TRP channel, TRPM3, a structural and functional analysis of d-opioid receptor, and identification of new ligands for DAF-12, a nuclear hormone receptor in *C. elegans*.

New Strategy for Defeating Malaria

Malaria, a disease caused by *Plasmodium* parasites, continues to be a serious health and societal burden in developing countries. Although there are number of treatment options for malaria, resistance to these treatments has been emerging, highlighting the need to identify new viable drug targets. One such target is the *Plasmodium* N-myristoyltransferase (NMT). NMTs catalyze transfer of myristate (Myr) from Myr-CoA to substrate proteins and are found in all eukaryotes. Members of *Plasmodium* genus express a single NMT enzyme that was previously proposed to be a potential drug target. However, questions about *Plasmodium* NMT function, the nature and number of its substrates, and the effect of NMT inhibition on the parasite physiology are wide open.

A recent report by Wright et al. (2013) significantly changes this situation. The authors combine chemical proteomic profiling, small molecule inhibitor development, biochemical and structural analysis, and testing using intraerythrocytic parasite cultures and an in vivo rodent model to provide answers to those questions. They identify more than 30 NMT substrates with a range of functions, including motility, invasiveness, and development. Additionally, the authors show that the two small molecules they developed lead to killing of blood-stage parasites via NMT inhibition in parasite cultures and that one of the small molecules exhibits promising in vivo activity in the mouse model. Furthermore, Wright et al. provide evidence that NMT inhibition prevents the innermembrane complex (IMC) assembly in asexual blood-stage parasites, thus contributing to parasite death. Overall, this work has important implications for studying NMT function and the role of N-myristoylation in *Plasmodium* and highlights opportunities for future antimalarial drug development.



The two classes of malaria parasite NMT inhibitors identified by the authors share major binding determinants but traverse the binding site by different trajectories. Image courtesy of E. Tate, M.D. Rackham, and J.A. Brannigan.

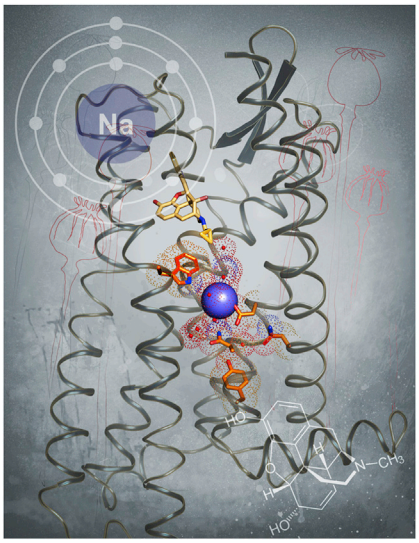
Wright, M.H., et al. (2014) Nat. Chem. 6, 112–121.

An Alternate Route in the TRP Channel

Transient receptor potential (TRP) channels are a family of ion channels with diverse physiological roles, from sensing pain to sensing wasabi. TRP channels share the basic tetramer architecture and gating and conduction mechanisms with a larger superfamily of cation channels. Recently, evidence emerged that cation channels baring specific mutations have an alternative route for cations to use, distinct from the central cation-conducting pore.

Vriens et al. report that such an alternative pathway exists and functions in a TRP channel, TRPM3, which, among other things, plays a role in aversive and nocifensive behavior responses to neurosteroid pregnenolone sulfate (PS). First, the authors saw that combined use of PS and an antifungal drug, clotrimazole (Clt), leads to significant potentiation of TRPM3 currents. Interestingly, when Clt was applied at a concentration of 1 μM or higher, the authors noticed significant inwardly rectifying current under strongly hyperpolarizing potentials. After ruling out that the effect might be due to Clt-induced change of channel selectivity or through indirect effects, the researchers focus on providing evidence for the existence of alternative permeation pathway. Vriens et al. show that Clt leads to the change in PS-induced cation influx by promoting Na^+ influx over Ca^{2+}, that blocking the outward current does not inhibit the inward one, and that two distinct types of currents are seen at the single channel level, which all suggests that inwardly rectifying current uses a path different from the central cation-conducting pore. Additional mutagenesis-based analysis and measurements in sensory neurons further support this conclusion. Importantly, in vivo tests done in mice point to the possibility that Clt-induced alternative cation pathway results in enhanced pain response to PS. This may have implications for the molecular basis of known Clt side effects and points to the possibility that an alternative cation route might exist in other TRP channels.

Vriens et al. (2014). Nat. Chem. Biol. Published online January 5, 2014. http://dx.doi.org/10.1038/nchembio.1428.



The 1.8 Å resolution structure of the δ-opioid receptor (grey cartoon) showing the sodium ion (blue sphere) and some of the "efficacy switch" residues (orange sticks) in the receptor allosteric site. Smaller red spheres are waters in the first and second coordination shells of the sodium ion. The morphinan derivative ligand naltrindole is represented by yellow sticks. The chemical structure of morphine is also shown. Image courtesy of Katya Kadyshevskaya in the R.C. Stevens lab at The Scripps Research Institute.

δ-Opioid Receptor: Watch for the Sodium

Opioid receptors (OR) are members of the G protein-coupled receptor (GPCR) family that respond to endogenous and exogenous opioid ligands, as well as a growing number of small molecule agonists. It is known that agonists exert their effect on ORs by binding to an orthosteric site, although mechanistic details at a molecular level are still unclear.

In a recent study, Fenalti et al. describe a high-resolution crystal structure of the human δ-OR in complex with naltrindole, a selective δ-OR anatagonist, which led to the discovery of an unexpected allosteric Na^+ site. To facilitate crystallization, the authors used a carefully prepared construct in which segments of the N and C termini of human δ-OR were removed and the thermostabilized *E. coli* apocytochrome b_{562}RIL (BRIL) fused to the truncated N terminus. The resulting construct crystallized from the lipid cubic phase and diffracted to 1.8 Å resolution. The construct's organization and the improved resolution over the available structure of mouse δ-OR fused to T4 lysozyme enabled a clearer view of the intracellular loop 3, the orthosteric binding pocket, and the previously mentioned allosteric Na^+ site to emerge. Interestingly, the structure captures δ-OR in inactive state conformation in which the Na^+ ion forms a connectivity network that involves both δ-OR residues and a number of water molecules and likely serves to enable communication between orthosteric and allosteric sites. Follow-up studies using point mutations of Na^+ site residues show that allosteric Na^+ anchoring residues act as efficacy switches and redirect δ-OR signaling from the $G\alpha_i$ protein pathway to a noncanonical β-arrestin pathway. This has interesting implications for δ-OR signaling, physiology, and pharmacology.

Fenalti et al. (2014). Nature. Published online January 12, 2014. http://dx.doi.org/10.1038/nature12944.

New Ligands for DAF-12

Nuclear hormone receptors (NHRs) are transcription factors regulated by small molecules, for example steroid hormones. They orchestrate expression of large number of genes to control diverse cellular processes such as development and aging. In simple terms, ligand binding leads to conformational change that modulates NHR interaction with co-activator and co-repressor proteins and leading to specific changes in transcription of target genes. Some NHRs bind more than one ligand, and different ligands are known to elicit different responses. Unfortunately, our current map of the entire NHR ligand space contains significant gaps, even in well studied models such as *C. elegans* or mouse.

The recent efforts of Mahanti et al. are aimed at filling out some of these gaps and identifying additional ligands for the most prominent NHR in *C. elegans*, DAF-12. The authors combine an NMR-based metabolomics strategy, DANS (differential analyses by 2D NMR spectroscopy), which provides an unbiased view of the metabolome, with specific genetic manipulation of *C. elegans,* which introduces defined perturbations in the biosynthetic pathway known to produce DAF-12 ligands. Comparison between wild-type and mutant metabolomes led to identification of several steroid molecules with unusual structures: (25*S*)-3-oxo-1,7-cholestadienoic acid, (25*S*)-3-oxo-7-cholestenoic acid, and (25*S*)-3α-hydroxy-7-cholestenoic acid. The authors show that these metabolites are DAF-12 ligands and that they act through DAF-12 to regulate developmental progression and *C. elegans* lifespan. Additionally, Mahanti et al. examine biosynthesis of DAF-12 ligands and show that it is tissue-specific and that ligands may be produced by nonidentical pathways and at different levels at different life stages. This work highlights the diversity of NHR ligand chemical space and the need to use sophisticated analytical methods to reinvestigate mammalian NHR ligand biosynthesis and NHR physiology, which play major roles in regulating human development and metabolism.

Mahanti et al. (2014). Cell Met. 19, 73–83.

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